

# The Role of Growth of Normal and Preneoplastic Cell Populations for Tumor Promotion in Rat Liver

by R. Schulte-Hermann,\* J. Schuppler,†  
I. Timmermann-Trosiener,\* G. Ohde,\*  
W. Bursch\* and H. Berger\*

A number of different compounds, including phenobarbital, hypolipidemic drugs such as clofibrate and nafenopin, the sex steroids progesterone, cyproterone acetate, estradiol and mestranol, chlorinated hydrocarbons such as DDT, hexachlorocyclohexane, and TCDD and the antioxidant butylhydroxytoluene, appears to promote the development of liver tumors from previously induced initiated cells. The mechanisms of tumor promotion by several representative prototypes of these compounds were studied in rat liver *in vivo*.

All liver tumor promoters mentioned above stimulate growth of normal liver. The growth response is due to cellular hypertrophy and/or increased rate of DNA (and cell) replication and/or decreased rate of cell death.

Hepatocytes in foci or islands of altered cells (putatively preneoplastic) show higher rates of replication than normal liver cells; various different liver tumor promoters cause a further increase of proliferation of focal cells. The increased proliferative activity is found in different island phenotypes and thus seems to be a useful marker of the putative preneoplastic state. The focal cells respond to several factors limiting proliferation in normal liver, suggesting that they are not autonomous with respect to growth control.

Early preneoplastic foci grow slowly without promotion, despite the relatively high rates of cell replication. Thus their cells seem to have a much shorter life-time than normal hepatocytes or to undergo reversion to the normal phenotype. Promoters seem to accelerate island enlargement by increasing cell replication and delaying cell death or remodeling. Thus, tumor promoters enhance the manifestation of the proliferation advantage of the putative initiated cell population.

In addition, promoters cause increases in the number of detectable islands. This can partially be explained by enlargement of existing islands, but phenotypic changes that would enhance the probability of detection of remodelling islands and growth of dormant initiated cells, probably contribute to the apparent increase of island number.

Putative preneoplastic foci of unknown origin are frequent in the liver of aged Wistar rats. They are morphologically and functionally very similar to those induced by carcinogens and are responsive to the mitogenic effect of tumor promoters. Promotion of these "spontaneous" foci may explain tumor appearance after long-term application of promoters.

The findings may provide a basis for improved identification of initiated hepatocytes (and of initiating hepatocarcinogens) and for detection of tumor promoters. All suspected liver tumor promoters tested so far induced enhanced preneoplastic cell proliferation after single doses. The long-term carcinogenicity bioassay as currently performed does not discriminate between initiating and promoting properties of a test compound if the animals used develop spontaneous preneoplastic lesions in the organ affected.

## Introduction

A variety of compounds has been shown to accelerate the development of liver tumors in two-stage experiments, in which an initiating carcinogen is given first, followed by long-term application of the

\*Institut für Toxikologie und Pharmakologie der Philipps-Universität, Pilgrimstein 2, 3550 Marburg, West Germany

†Department Toxikologie Schering AG Berlin-Bergkamen  
Müllerstrasse, 1000 Berlin 65, Germany

compound itself. Examples are listed in Table 1. These findings suggested tumor-promoting activity of the test compounds (1-4). However, most of the agents were hepatotumorigenic also if given without pretreatment with an initiating carcinogen (5). Observations of this type cast serious doubts on the interpretation that the agents are (pure) tumor promoters and have even prevented the general acceptance of the initiation-promotion concept in chemical (liver) carcinogenesis. In fact, we have recently shown with the  $\alpha$ -isomer of hexachlorocyclohexane (HCH) as a model compound that discrimination between initiating and promoting properties can be impossible on the basis of the long-term carcinogenicity bioassay alone (4).

Obviously improved knowledge of the mechanisms of tumor promotion is required to develop methods for detection of promoting compounds which would be more reliable and more rapid than the classical long-term bioassay. Better understanding of tumor promotion would also appear to be necessary for reliable assessment of potential health risks resulting from use of promoting compounds. We will present a short overview on studies performed by us during the past several years on three questions pertinent to the mechanisms of tumor promotion in the liver: properties of tumor promoters relevant to the promoting action; properties of the initiated cells relevant to promotion; and how "pure" promoters (if they exist) may lead to tumor appearance in the liver.

Tumor promotion includes as an essential component the proliferation of the pool of initiated hepatocytes. Thus understanding the control of cell proliferation in normal liver and its disturbance in initiated cells is of crucial importance to the elucidation of the mechanism of tumor promotion. Therefore, some aspects of growth control in the liver will also be considered.

It should be noted that by definition complete he-

patocarcinogens and also toxic agents such as  $\text{CCl}_4$  possess tumor-promoting activity in the liver. Effects of these agents are not considered in the present paper.

## Methods

Female Wistar rats (Zentralinstitut für Versuchstierzucht, Hannover, Germany) were used at an initial age of 4 to 8 weeks unless stated otherwise. Where indicated, the animals were adapted for 3 weeks to a controlled lighting rhythm (light-dark 12-12) and to a daily feeding period of 5 hr. This regimen served to synchronize endogenous and mitogen-induced DNA synthesis in the liver (6). For induction of putative preneoplastic foci, 75 or 150 mg/kg diethylnitrosamine (DNA) or 250 mg/kg *N*-nitrosomorpholin (NNM) was dissolved in water and administered as single oral doses. Hepatomitogenic/promoting compounds were given as follows: Phenobarbital (PB) orally,  $1 \times 50$  mg/kg in aqueous solution or daily 50 mg/kg via the food;  $\alpha$ -hexachlorocyclohexane ( $\alpha$ -HCH) orally,  $1 \times 200$  mg/kg in oil or daily 20 mg/kg via the food; cyproterone acetate (CPA) orally in oil, 40 or 100 mg/kg once or daily or 100 mg/kg once weekly; progesterone (Pro) SC 500 mg/kg once weekly; ethinylestradiol ( $\text{EE}_2$ ) SC in oil/benzyl benzoate 0.5 mg/kg daily.  $^3\text{H}$ -thymidine (0.2 mCi/kg) was injected IV to measure DNA synthesis; clock times of injection were scheduled to the period of maximal DNA synthesis during the daily rhythm (6).

After decapitation, specimens of liver tissue were fixed in formalin or cold acetone, and histological sections were prepared by standard procedures and stained with hematoxylin and eosin or were assayed for  $\gamma$ -glutamyltransferase ( $\gamma$ -GT) activity (7). Autoradiography was performed on the same sections to measure DNA synthesis, which was determined by counting labeled cells ( $^3\text{H}$ -index = percent

Table 1. Compounds believed to promote tumor development in rodent liver and their acute hepatic effects.

Class	Compound <sup>a</sup>	Increased synthesis of	Proliferation of <sup>b</sup>	Growth
Sedatives	Phenobarbital	Monooxygenase (PB-type)	SER	+
Estrogens	Estradiol	Various serum proteins		+
Progestins	Ethinylestradiol/mestranol			
	Progesterone	Monooxygenase (PCN-type)	SER	+
	Cyproteronacetate (= CPA)			
Hypolipidemic drugs	Nafenopin, Clofibrate	Enzymes of fatty acid metabolism	Microbodies	+
Chlorinated hydrocarbons	Hexachlorocyclohexane (= HCH), DDT, PCB's, TCDD	Monooxygenase (PB, MC-type)	SER	+
Antioxidants	Butylhydroxytoluene	Monooxygenase	SER	+

<sup>a</sup>PB = phenobarbital; PCN = pregnenolone-16  $\alpha$ -carbonitrile; MC = 20-methylcholanthrene.

<sup>b</sup>SER = smooth endoplasmic reticulum

of hepatocytes labeled). Biochemical determination of DNA content and of the specific activity of DNA was done as described previously (6, 8) using the procedure of Burton (9).

## Results and Discussion

### Effects of Tumor Promoters in Normal Liver

A central question in tumor promotion concerns the properties of tumor promoters relevant to the promoting action. The biological effects of putative tumor promoters in normal liver might provide a first clue to identify the critical properties. As is obvious from the compilation shown in Table 1, liver tumor promoters tentatively identified so far are extremely heterogeneous with respect to chemical structures and general pharmacological or biological effects. They share, however, the ability to induce in the liver increases of specific functions and growth, often considered to be adaptive responses to the agent. Functional changes include increased synthesis of certain serum proteins or of enzyme families such as drug-metabolizing enzymes or enzymes of the fatty acid metabolism. These changes are frequently accompanied by multiplication of specific organelles such as smooth endoplasmic reticulum (SER) or microbodies. The third component of the adaptive response is the induction of liver growth (5, 10). Indeed, stimulation of liver growth appears to be the only known effect common to all the various tumor promoters listed in Table 1. Likewise, in various other organs, compounds promoting tumor development also stimulate growth of that organ. It therefore seemed justified to adopt the hypothesis that the ability to induce growth is one of the critical properties of tumor promoters. Further evidence supporting this hypothesis will be presented below.

How is the growth response of the liver brought about? With most of the compounds mentioned in Table 1 it is due to a combination of cellular hypertrophy and hyperplasia, the contribution of which to the total enlargement of the liver varies considerably depending on the specific compound and the experimental conditions (5, 10). As an example, Figure 1 depicts the effects of ethinylestradiol (EE<sub>2</sub>) on the liver. This estrogen is most frequently used in human contraceptive formulations; its effects on liver growth were studied recently in some detail (11). That estrogens may cause growth of rodent liver was observed several years ago (12), but no attention was paid to these findings. As shown in the upper part of Figure 1 the effect of EE<sub>2</sub> on liver size is moderate if compared to controls fed *ad libitum*, and this may be the reason why the profound stimulatory effect of estrogens on liver growth have been

overlooked by most investigators. Estrogens decrease food consumption, and this results in a decrease of liver size (Fig. 1). Thus, if liver mass after EE<sub>2</sub> treatment is compared to that in pair-fed control animals, a very pronounced increase of about 80% is seen. This increase is accompanied by a steep increase of hepatic DNA synthesis, with a peak on the first day after start of treatment and a subsequent decline almost back to control values during the ensuing seven days. This increase which by histological-autoradiographic studies has been found to be predominantly due to parenchymal DNA synthesis results in a considerable increase of hepatic DNA content (Fig. 1, lower part). Mitotic activity is also increased (data now shown.)

It should be noted that the increase of DNA synthesis declines almost to control values even though EE<sub>2</sub> treatment is continued. The same has been found with cyproterone acetate (CPA) (Fig. 2), and with all other hepatomitogens/tumor promoters tested so far (5, 8, 10). Obviously an effective feedback mechanism prevents excessive accumulation of DNA in response to mitogenic stimuli. Figure 2 also presents another important aspect of mitogen action. It can be seen that the increased content of DNA persists as long as the treatment is preformed. However, if the treatment is stopped, the DNA content of the liver declines rapidly in a matter of a

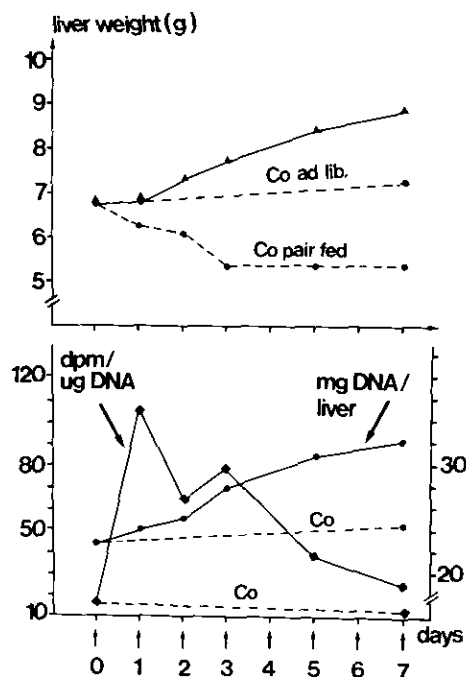


FIGURE 1. Effects of ethinylestradiol on size, DNA content and DNA synthesis of rat liver. Arrows: treatment with 0.5 mg/kg EE<sub>2</sub>.

few days and then more slowly until eventually the control level will be reached. It appears that in the hyperplastic liver at least some of the cells depend on the continuous presence of the growth stimulus; its withdrawal causes rapid death of some of the cells (Fig. 2) (13). In summary, CPA as well as the other compounds studied so far appear to stimulate liver growth by hypertrophic and/or mitogenic effects on hepatocytes and to maintain cell number at an enhanced level by preventing cell death. These effects are well known actions of various trophic hormones in their target organs, and it may be concluded that the liver mitogens/promoters studied have a hormonelike effect on the normal liver.

### Short-Term Effects of Promoters on Proliferation in Foci of Altered Cells

A second question central to understanding tumor promotion concerns identification and properties of the target cell of promoters from which tumors eventually arise. According to the two-stage concept, this cell should be the initiated cell, which unfortunately has not yet been identified in the liver or other organs. There is, however, rather strong evidence suggesting that foci or islands of phenotypically altered cells appearing in the liver of carcinogen-treated animals are the immediate

progeny of the initiated cell. Consequently we have studied the effect of the tumor promoters on these altered, putatively preneoplastic (pn) cells, and particular attention was paid to the growth behavior of these cells. Rats were treated with single doses of DENA or NNM, and after 3 weeks to 11 months, islands were identified by means of a positive  $\gamma$ -glutamyltransferase ( $\gamma$ GT) reaction, increased basophilia or clearness of cytoplasm (14-16).

It is important to note that this regimen of initiation results in appearance of neoplastic nodules or carcinomas in the liver only after more than 1 or 2 years, if no promoters are applied. The pn lesions induced thus appear to be in an early stage of tumor development. Invariably the island cells exhibited higher rates of DNA synthesis and mitotic activity than the normal hepatocytes (Fig. 3). Obviously the rate of proliferation within the islands is above the needs for homeostasis of cell number in the whole liver. Single doses of various types of tumor promoters resulted in a further increase of the already enhanced proliferation activity of island cells. The increased proliferative activity was found in different island phenotypes (17) and thus seems to be a marker more consistently associated with the putative pn state than the morphological and histochemical alterations often used.

### Long-Term Effects of Promoters on Islands of Altered Cells

Islands of altered cells exhibited increased rates of DNA synthesis at all times investigated between three weeks and 11 months after administration of

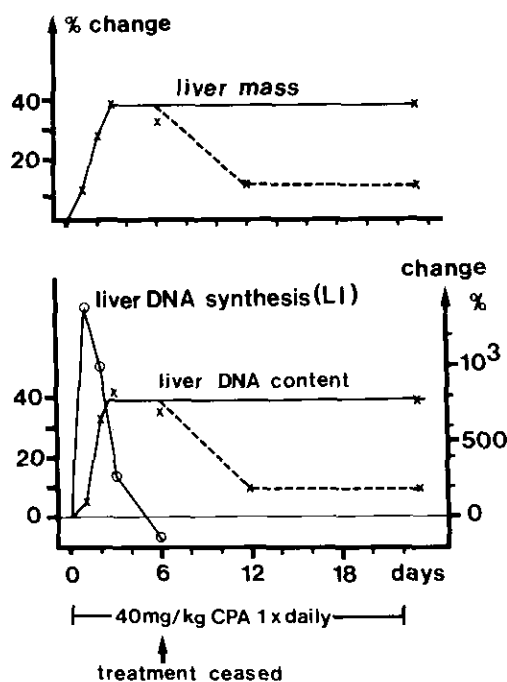


FIGURE 2. Effects of cyproterone acetate treatment and withdrawal on DNA content and DNA synthesis of rat liver. Daily dose, 40 mg/kg CPA

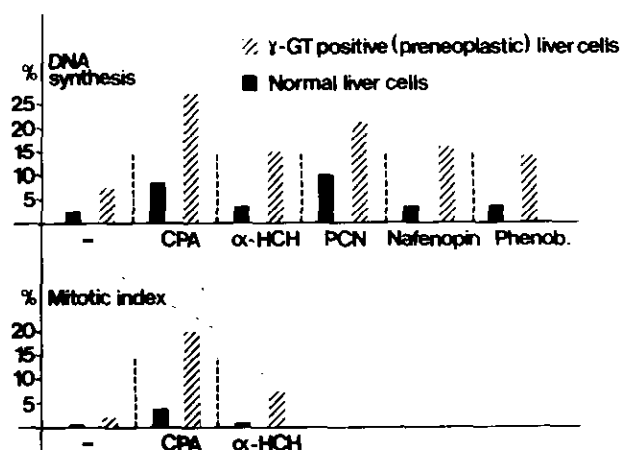


FIGURE 3. DNA synthesis and mitotic activity in foci of  $\gamma$ -GT positive cells following single doses of various suspected liver tumor promoters. PCN = pregnenolone-16 $\alpha$ -carbonyl, 100mg/kg; nafenopin: 100 mg/kg; CPA: 100 mg/kg; other doses, see text.

the carcinogen (Figs. 4 and 5). Assuming an average proliferation rate of 1.5%/day, islands should show rapid growth even without promotion with a doubling time of about 45 days (Fig. 5). However, the growth rate of the average island actually found was almost zero for several months and then increased gradually (Figs. 4 and 5). Thus it appears that the altered cells have a life-time shorter than that of normal hepatocytes, which is several hundred days. Alternatively, the island cells may "re-model" to a phenotype indiscriminable from normal hepatocytes.

Long-term effects of promoters on island developments were studied in two different experimental designs. In the first design, two suspected tumor promoters, i.e., CPA and progesterone (Pro), were given intermittently once per week; in the other, phenobarbital or  $\alpha$ -HCH was administered continuously via the food.

Intermittent treatment with Pro or CPA caused strikingly enhanced DNA synthesis rates in islands at each time investigated. The upper part of Figure 4 shows, as an example,  $^3\text{H}$ -indices found after 6 months of treatment. This effect was associated

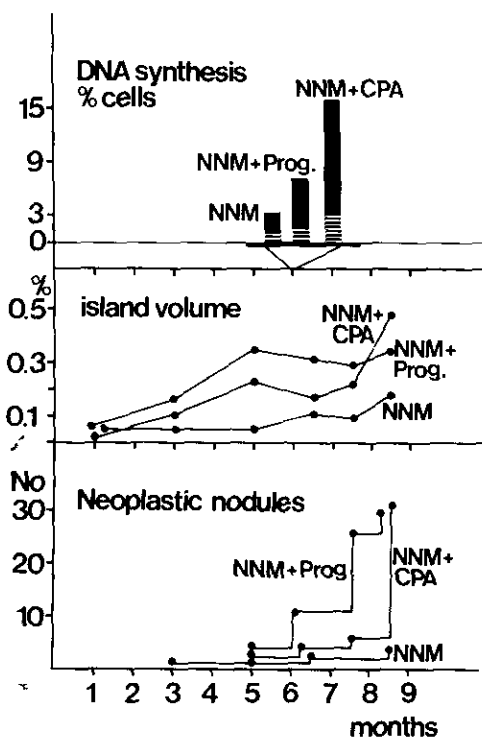


FIGURE 4. Effects of intermittent treatment with cyproterone acetate and progesterone on island growth and tumor appearance following initiation by a single dose of NNM. Promoter treatment was started at time "0". Hatched columns: normal hepatocytes; black columns:  $\gamma$ -GT positive island cells.

with increases in the average island volume (Fig. 4, middle part). Island enlargement precedes the appearance of neoplastic nodules in the hormone-treated rats (Fig. 4, lower part). These findings strongly suggest that CPA and Pro promote tumor development in rat liver and that hormone-induced increases of island cell proliferation are at least partially responsible for island enlargement under the present conditions. Moreover, the findings are consistent with the hypothesis that island enlargement is a rate-limiting factor in liver tumorigenesis. In addition, we noted an increase in the number of detectable islands (18).

Results obtained after continuous treatment with phenobarbital are presented in Figure 5. Since the effects of  $\alpha$ -HCH were qualitatively very similar

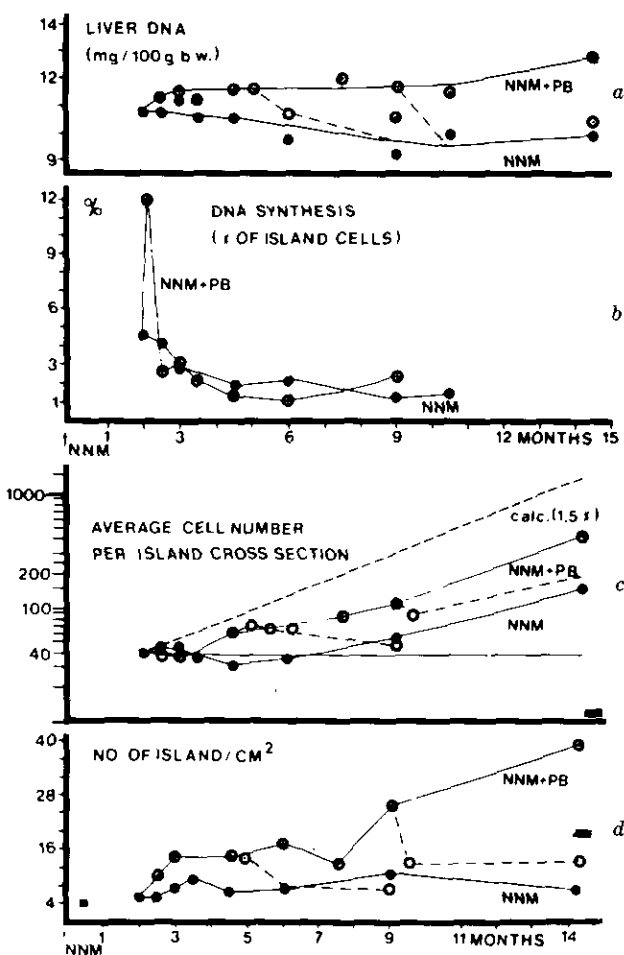


FIGURE 5. Effects of continuous treatment with phenobarbital on (a) liver DNA, (b) island DNA synthesis, (c) island size and (d) island number following initiation by a single dose of NNM administered at zero time. PB treatment started 2 months after NNM. (—) theoretical island growth rate assuming cell replication of 1.5%/day.

though somewhat more pronounced with respect to all parameters studied they are not shown. The hepatic content of DNA was increased at all times investigated (Fig. 5a). DNA synthesis in normal hepatocytes, after a small initial rise, was essentially the same as in untreated rats as expected (data not shown); in island cells it was increased initially but rapidly declined to the level found in islands of untreated rats (Fig. 5b). This suggests that DNA synthesis in the average island, though it remains at a higher level than in normal cells, is sensitive to feedback control of hepatocyte proliferation (8). Average size and number of islands both increased severalfold during phenobarbital treatment as observed before (19). While the increase in number already appeared in the early weeks of promotion, island enlargement was detectable only after some delay (Figs. 5c and 5d). The size distribution of islands after 28 weeks of treatment with phenobarbital or  $\alpha$ -HCH is shown in Figure 6.

How can these findings be explained? It appears that increased island cell proliferation cannot be the only factor responsible for island enlargement during continuous exposure to  $\alpha$ -HCH and phenobarbital. We therefore assume that under these conditions island growth is due to a decrease in the rates of cell death or phenotypic remodeling, either of which appears to occur in island cells (see above). This assumption seems reasonable since prevention of cell death has been observed to be one of the promoter effects in normal liver (Fig. 2) and since promoters have already been shown to prevent phenotypic changes in islands (20). The increased number of islands should partially result from island enlargement which increases the probability of detection, and partially from growth of miniislands and single initiated cells to a detectable size. This would

imply that a considerable number of initiated hepatocytes do not proliferate to form detectable islands unless a promoter is applied. In addition, promoters may possibly lead to phenotypic changes within existing but undetectable islands in a non-promoted liver in such a way that they are more clearly demarcated from the surrounding normal tissue.

These considerations are supported by findings obtained after withdrawal of PB (Fig. 5). The total DNA content of the liver returned to the control level although more slowly than in the experiment presented in Figure 2. In parallel, number and average size of islands returned to the levels found in nonpromoted animals (Figs. 5c, d). Some of the residual islands exhibited signs of phenotypic reversion to a more normal appearance, such as partial loss of  $\gamma$ -GT. Thus both remodeling of island cells to the normal phenotype and island cell death (suggested by the loss of liver DNA) may have contributed to the decreases of island size and number. In any event part of the island cells found after a period of promotion seem to be promoter-dependent for survival or specific phenotypic appearance. These results are compatible with the assumption that PB induces growth of a subpopulation of promoter-dependent islands. The basic defect in this subpopulation would be a persisting imbalance of cell proliferation vs. cell death/remodeling during prolonged promoter treatment when the normal liver has reached a new steady state of cell number (see above). This property seems to be different from another feature of putative initiated cells, i.e. resistance which is thought responsible for island growth during promotion with AAF (15, 21).

The regression of islands after promoter withdrawal is of considerable interest from a practical toxicological point of view, since it confirms previous findings made with skin tumors suggesting that the action of a promoter is reversible within certain limits.

We conclude that the ability to induce liver growth or the hormonelike effect is an essential, though not necessarily the only critical property of the tumor promoters. Island cells have a proliferation advantage over normal hepatocytes; tumor promoters seems to enhance the manifestation of this advantage. Properties of promoters and of putative preneoplastic islands relevant for tumor promotion and also of possible help for identification of both are listed in Tables 2 and 3.

### Growth Control in Foci of Altered Cells

By using the procedures described above it is possible to study features of growth control of putative preneoplastic hepatocytes. Despite of their in-

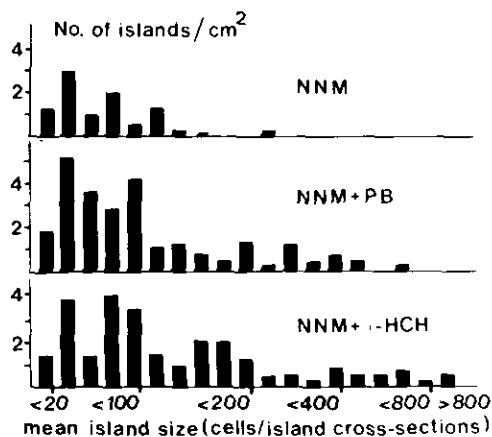


FIGURE 6. Island size distribution after 28 weeks of continuous treatment with phenobarbital and  $\alpha$ -HCH.

creased proliferative activity (see above), these cells are not autonomous with respect to growth control. First, their proliferation rate, although higher than in normal liver cells, is still much lower than in liver lesions further advanced towards the malignant state. Second, island cells appear to respond to feedback inhibition of DNA synthesis during prolonged promoter treatment (Fig. 5) as do normal liver cells (Figs. 1, 2). Third, island cells seem to depend as normal cells on permissive signals released by consumption of food: In normal hepatocytes, food consumption has been found to control the induction of cell replication by mitogens at two specific stages before and during the replicative cycle, i.e. in the  $G_0$  phase and in the R phase, several hours before initiation of DNA synthesis. Fasting in one of these phases makes the liver nonresponsive to the mitogenic effect of the agents. Thus in addition to the specific mitogenic signal exerted by the mitogens cell replication in the liver depends on the presence of permissive signals which are released after food consumption (Fig. 7) (6, 22). As shown in Figure 8, fasting in either the  $G_0$  or R state largely suppresses DNA synthesis in foci of altered cells similar as in normal liver cells. In conclusion, with respect to their proliferative behavior, island cells appear to be in a state between normalcy and malignancy. The basic defect which renders island cells more susceptible to endogenous and exogenous mitogenic stimuli is still unknown.

Table 2. Properties of liver tumor promoters.

Property
Induce of growth and functional changes in the liver (hormone-like effect)
Enhanced proliferation of putative preneoplastic cells
Accelerate growth of putative preneoplastic islands
Produce histochemical/morphological changes in putative preneoplastic islands
Accelerate appearance of tumors

Table 3. Properties of cells in islands of altered (initiated) hepatocytes which are relevant to island growth.

Property
Proliferation rate in excess of homeostatic need of the whole liver
Short lifespan and/or phenotypic instability
Response to liver tumor promoters:
Increase of proliferation rate
Increase of lifespan and/or prevention of phenotypic reversion
Sensitivity to feedback-inhibition of cell proliferation
Dependence on permissive signals exerted by food consumption

## Progression

If some or all of the foci of altered cells are potential intermediates on the pathway to cancer, progression to more malignant phenotypes should occasionally occur within islands. This has been observed by others (21) and also in the present study. Progression may be due to a (rare) event similar to that causing initiation (21). On the basis of our find-

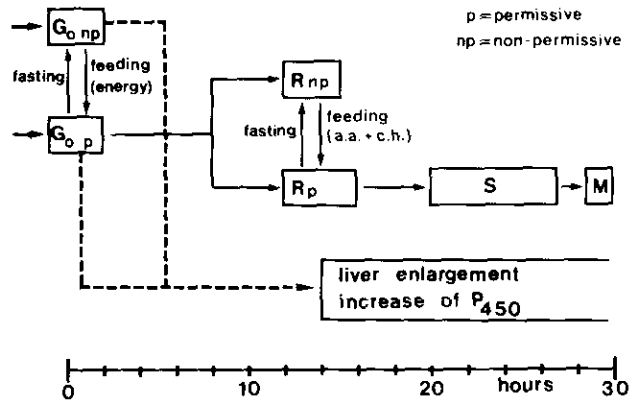
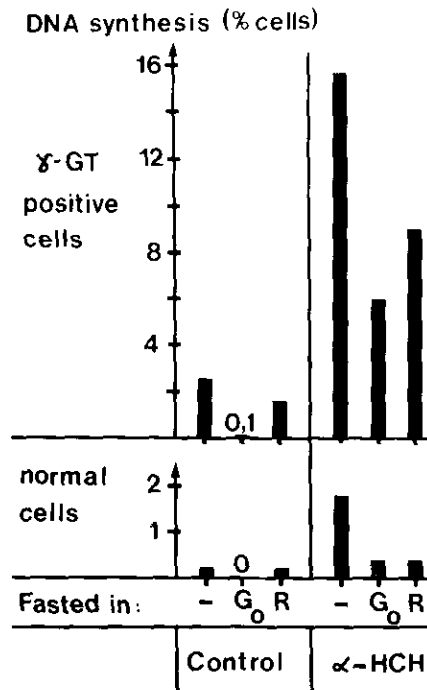


FIGURE 7. Scheme showing interactions between feeding and hepatomitogens in the control of the hepatic replicative cycle. a.a. = amino acids; c.h. = carbohydrates.

FIGURE 8. Effect of fasting during the  $G_0$  and R phase of the replicative cycle on DNA synthesis in foci of altered hepatocytes.

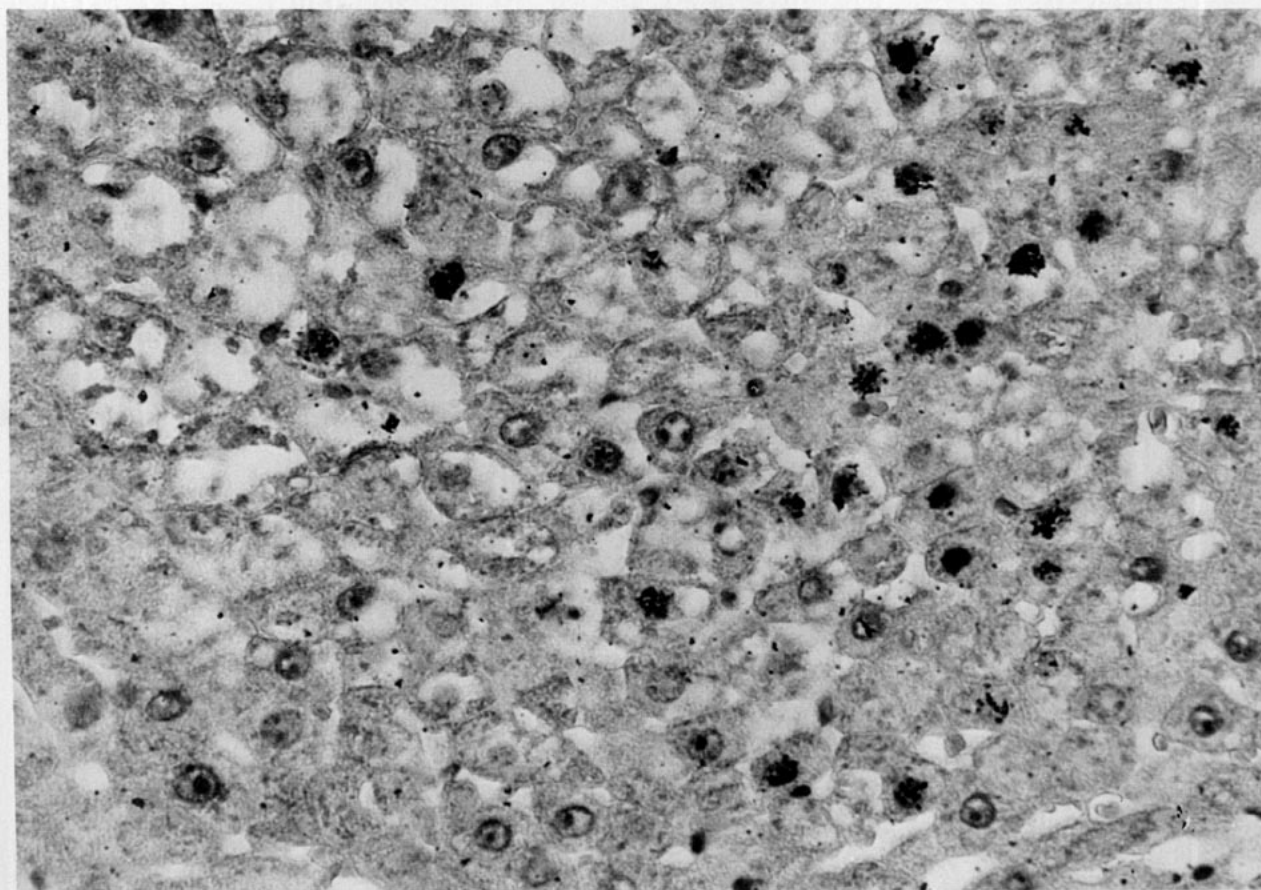
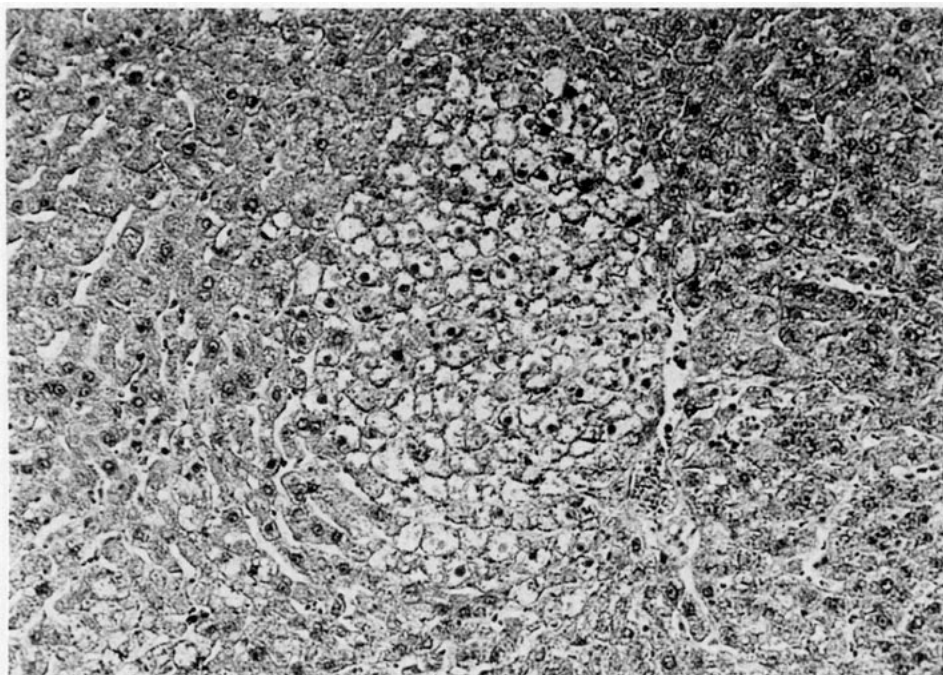


FIGURE 9. Islands of altered cells in the liver of an untreated 2-year-old Wistar rat: (top)  $25\times$ ; (bottom)  $180\times$ . Note the presence of numerous island cells labelled with silver grains indicating DNA replication.



ings we propose the hypothesis that the relatively high replicative activity of island cells may be responsible for progression. Since cell replication seems necessary to fix any damage potentially leading to initiation (or progression), the chances of fixation of critical damage are much higher in islands than in normal liver. Moreover, replication itself carries a certain risk of errors which occasionally may lead to progression. In the adult rat, the frequency of abnormal, erroneous mitoses reportedly is high even in the noninitiated liver (23).

Due to the increased proliferative activity, an island liver cell may replicate about 10 times a year, while a normal liver cell would replicate approximately once. Thus the average island cell should have a much higher risk of initiationlike phenotypic changes than the normal hepatocyte. Multiplication and enlargement of islands during promotion dramatically expands the population "at risk;" as a result, a rare event leading to progression is much more likely to occur at an early time than in nonpromoted liver.

### Effect of Promoters on Islands Appearing Spontaneously

As mentioned above, tumors may occasionally develop after sole treatment with a promoter without initiating pretreatment. This finding has similarly been made with the promoters *par excellence*, i.e., the phorbol esters, in studies on skin tumorigenesis and has supported doubts expressed by several workers on the validity of the promotion concept. The defenders of the concept explained the formation of tumors by promoters alone by assuming promotion of spontaneous initiated cells, but to our knowledge this claim has not yet found much experimental support.

We have searched for putatively initiated cells in the liver of 2-year-old Wistar rats, using for identification the proliferative characteristics of carcinogen-induced island cells, in addition to the usual morphological and histochemical markers. Although the Wistar substrain used has a low background of spontaneous hepatoma (2%), all of 41 animals studied had in the liver islands of altered cells which on the basis of histochemical, morphological and functional criteria (increased cell proliferation) were very similar if not identical to those produced in young animals by application of hepatocarcinogens (Fig. 9). As shown in Figure 10 these "spontaneous" islands exhibit an increased rate of DNA synthesis over that of normal hepatocytes. Most importantly, various different liver tumor promoters cause a further increase of the DNA replication rate; this shows that the "spontaneous" islands are respon-

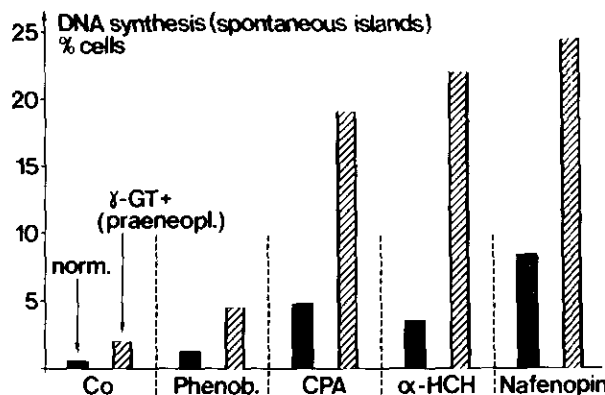


FIGURE 10. DNA synthesis in "spontaneous" foci of altered cells: (Black) normal cells; (cross-hatched)  $\gamma$ -GT positive islands. Treatment: see text, methods and Fig. 3.

sive to the mitogenic effects of the tumor promoters. Prolonged application of the promoters therefore should result in rapid island growth and eventually in tumor formation.

This finding implies that long-term carcinogenicity bioassays of chemical agents do not discriminate between initiating and promoting properties of a test compound, if preneoplastic lesions develop spontaneously in the affected organ. Obviously additional tests are required for reliable interpretation of data and risk assessment. The properties of tumor promoters listed in Table 2 would appear to provide useful parameters for the detection of promoting agents. In particular the observed enhancement of proliferation of island cells in response to tumor promoters might provide a useful and reliable shortterm test *in vivo*. This possibility will be checked in further studies.

In view of the ubiquitous presence of a vast number of carcinogenic factors in our environment the formation of initiated cells may be a frequent event in the liver and in other organs of animals and humans. According to some current evaluations of long-term carcinogenicity, a number of drugs and other chemicals including various natural and synthetic hormones have been classified as (initiating) carcinogens. However, several of these agents induce growth and promote tumor development in their target organs. On the basis of the present results it is tempting to consider the possibility that promotion of spontaneous initiated cells may explain the development of cancer by nonmutagenic drugs and hormones in various organs of animals and humans.

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